



US005976567A

United States Patent [19]

Wheeler et al.

[11] Patent Number: 5,976,567

[45] Date of Patent: *Nov. 2, 1999

- [54] **LIPID-NUCLEIC ACID PARTICLES
PREPARED VIA A HYDROPHOBIC LIPID-
NUCLEIC ACID COMPLEX INTERMEDIATE
AND USE FOR GENE TRANSFER**

- [75] Inventors: **Jeffery J. Wheeler**, Richmond; **Marcel B. Bally**, Bowen Island; **Yuan-Peng Zhang**, Vancouver; **Dorothy L. Reimer**, Vancouver; **Michael Hope**, Vancouver; **Pieter R. Cullis**, Vancouver; **Peter Scherrer**, Vancouver, all of Canada

- [73] Assignee: **Inex Pharmaceuticals Corp.**, Vancouver, Canada

- [*] Notice: This patent is subject to a terminal disclaimer.

[21] Appl. No.: **08/660,025**

[22] Filed: **Jun. 6, 1996**

Related U.S. Application Data

- [63] Continuation-in-part of application No. 08/484,282, Jun. 7, 1995, Pat. No. 5,705,385, and a continuation-in-part of application No. 08/485,458, Jun. 7, 1995.
- [51] **Int. Cl.⁶** **A61K 9/127**; A61K 31/70; C12N 15/88
- [52] **U.S. Cl.** **424/450**; 435/458; 514/44
- [58] **Field of Search** 514/44, 2; 424/450; 435/172.3, 458; 935/54

[56] References Cited

U.S. PATENT DOCUMENTS

4,394,448	7/1983	Szoka, Jr. et al.	435/172.3
4,438,052	3/1984	Weder et al.	264/4.6
4,515,736	5/1985	Deamer	424/1.21
4,598,051	7/1986	Papahadjopoulos et al.	435/7.25
4,897,355	1/1990	Eppstein et al.	435/172.3
5,013,556	5/1991	Woodle et al.	424/450
5,171,678	12/1992	Behr et al.	435/172.3
5,208,036	5/1993	Eppstein et al.	424/450
5,225,212	7/1993	Martin et al.	424/450
5,264,618	11/1993	Felgner et al.	560/224
5,279,833	1/1994	Rose	424/450
5,283,185	2/1994	Epanet et al.	435/172.3
5,320,906	6/1994	Eley et al.	428/402.2
5,545,412	8/1996	Eppstein et al.	424/450
5,705,385	1/1998	Bally et al.	435/320.1

FOREIGN PATENT DOCUMENTS

WO 91/16024	10/1991	WIPO	.
WO 93/05162	3/1993	WIPO	.
WO 93 12756	7/1993	WIPO	.
WO 95/02698	1/1995	WIPO	.
WO 96/10390	4/1996	WIPO	.

OTHER PUBLICATIONS

- Puyal et al., Eur. J. Biochem. 228:697–703 (1995).
 Enoch et al. “Formation of 1000-Å-diameter, single-bilayer phospholipid vesicles” Proc. Natl. Acad. Sci. USA 76(1): 145–149, Jan. 1979.

Szaka et al. “Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation” Proc. Natl. Sci. USA 75(9): 4194–4198, Sep. 1978.

Hawley-Nelson, et al. Focus 15(3):73 (1993) Lipofectamine¹⁹⁸ Reagent: A New, Higher Efficiency Polycationic Liposome Transfection Reagent.

Stamatatatos, et al., Biochemistry 27:3917–3925 (1998), Interactions of Cationic Lipid Vesicles with Negatively Charged Phospholipid Vesicles and Biological Membranes. Leventis, et al., Biochem. Biophys. Acta 1023:124 (1990), Interactions of Mammalian Cells with Lipid Dispersions Containing Novel Metabolizable Cationic Amphiphiles.

Ballas, et al., Biochim. Biophys. Acta 939:8–18 (1998). Behr, Acc. Chem. Res. 26:274–78 (1993).

Culver, Gene Therapy: A Handbook for Physicians, Mary Ann Liebert, Inc. publishers, pp. 33–40 (1994).

Felgner, et al. Proc. Nat'l Acad. Sci, USA 84:7413–7417 (1987).

Wilson, et al., Biochemistry 18:2192–2196 (1979).

Duzgunes, Subcellular Biochemistry 11:195–286 (1985).

Szoka et al., Ann. Rev. Biophys. Bioeng. 9:467–508 (1980).

Legendre, Pharm. Res. 9:1235–1242 (1992).

Gao, et al., Biochem. Biophys. Res. Comm. 179:280–285 (1991).

Woodle, et al., Biochim. Biophys. Acta 1105:193–200 (1992), Versatility in Lipid Compositions Showing Prolonged Circulation with Sterically Stabilized Liposomes.

Juliano, Biochem. Biophys. Res. Commun. 63:651–658 (1975).

Zhu, et al., Science 261: 209–211 (1993), Systemic Gene Expression After Intravenous DNA Delivery Into Adult Mice.

Hyde et al., Nature 362:250–256 (1993), Correction of the ION Transport Defect in Systic Fibrosis Transgenic Mice by Gene Therapy.

Gershon, et al. Biochemistry 32:7413–7151 (1993).

Brigham, et al., Am. J. Med. Sci. 298:278–281 (1989), Rapid Communication: in Vivo Transfection of Murine Lungs with A Functioning Prokaryotic Gene Using A Liposome Vehicle.

Primary Examiner—Nancy Degen

Assistant Examiner—Thomas G. Larson

Attorney, Agent, or Firm—Townsend and Townsend and Crew

[57] ABSTRACT

Novel lipid-nucleic acid particulate complexes which are useful for in vitro or in vivo gene transfer are described. The particles can be formed using either detergent dialysis methods or methods which utilize organic solvents. Upon removal of a solubilizing component (i.e., detergent or an organic solvent) the lipid-nucleic acid complexes form particles wherein the nucleic acid is serum-stable and is protected from degradation. The particles thus formed have access to extravascular sites and target cell populations and are suitable for the therapeutic delivery of nucleic acids.